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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Marc H. Hedrick et al.	
Serial No.:	not yet known	Examiner: not yet known
Filed:	herewith	Group Art Unit: not yet known
Title:	ADIPOSE-DERIVED STEM CELLS AND LATTICES	

35 North Arroyo Parkway
Pasadena, California 91103
September 10, 2001

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

PRELIMINARY AMENDMENT

Please amend the subject application as follows.

IN THE CLAIMS:

Please cancel claims 8-138 without prejudice to pursue the subject matter of these claims in a continuation application to be filed in the future.

No fee is deemed necessary in connection with the filing of this Preliminary Amendment. If any fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

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In the IPEA/US

International Appl'n No. PCT/US00/06232	International filing date *(day/month/year)* 10 MARCH 2000 (10.03.00)	(Earliest) Priority date *(day/month/year)* 10 MARCH 1999 (10.03.99)
Title of invention ADIPOSE-DERIVED STEM CELLS AND LATTICES		
Applicant(s) UNIVERSITY OF PITTSBURGH OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION et al.		

Response to Written Opinion

Commissioner of Patents and Trademarks
BOX PCT
Washington, D.C. 20231

Attn: William Sandals

Dear Mr. Sandals

In response to the Written Opinion, mailed on April 19, 2001, please consider the following remarks and enter the attached substitute sheets 28-32, replacing pages 28 and 29 of the application and serving to amend the claims.

Discussion of the Written Opinion

The Written Opinion has not been established with respect to claims 17-38, 49-72 and 76 because no international search report was generated with respect to these claims. Moreover, the opinion has not been established with respect to claims 5-14 or 20-76 because they are drafted in multiple-dependent form. With respect to claims that have been examined (i.e., claims 1-4, 15, 16, and 77-79), the Written Opinion alleges that all claims lack novelty in light of several references. Additionally, the Written Opinion alleges that the claims lack inventive step in light of any of these primary references in combination with several other secondary references.

Discussion of the Amendments

The application is amended to add claims 80-138. Claims 80-131 concern a lipo-derived stem cell substantially free of mature adipocytes, which can be cultured in DMEM + about 10% fetal bovine serum without differentiating, and which has two or more identified developmental phenotypes, as well as populations of such cells and methods of using such cells. Such claims are supported in the specification, for example on page 2, lines 34-36, page 4, lines 35-37, and generally throughout the specification). Claims 132-138 concern a method of isolating stem cells from adipose tissue and are supported in the specification, for example, from page 3, line 24, through page 4, line 2; see also Example 1. Accordingly, these claims add no new matter to the application.

Discussion of Novelty

The Written Opinion alleges that claims 1-4, 15, 16, and 77-79 lack novelty in light of eleven references, as follows:

Reference	Claims
Dani et al.	1, 2, 15, 16
Dodson et al.	1, 2, 15, 16
Hui-ling Su et al.	1, 2, 15, 16
Marko et al.	1, 2, 15, 16
Sorisky et al.	1-3, 15, 16
U.S. Patent 5,486,359	1-4, 15-16, 77-79
U.S. Patent 5,728,739	1, 15
U.S. Patent 5,827,740	1, 15
U.S. Patent 5,827,897	1, 15
WO 98/04682	1-4, 15-16, 77-79
Young et al.	1, 2, 15, 16

Claim 1 is drawn to a "lipo-derived stem cell," and each of the other rejected claims depends from claim 1. As stated in the specification, a "lipo-derived stem cell" is a stem cell that is isolated from adipose tissue (see, e.g., page 3, lines 24-32). A "stem cell," in turn, is a cell that has the capacity to develop into at least two discrete developmental pathways (see page 23, lines 5-6). Thus, for any reference to anticipate the claims, such reference must disclose a cell that has the capacity to develop into at least two discrete developmental pathways that is isolated from adipose tissue. None of the references disclose such subject matter. Instead, the references disclose the following:

Reference	Disclosure
Dani et al.	Discloses isolation of embryonic stem cells from undifferentiated blastocysts (see page 1279, column 2). Obviously, at such an early developmental stage, no adipose tissue has formed. Thus, the stem cells isolated by Dani ***could not possibly be lipo-derived***.
Dodson et al.	Disclosed a muscle/fat co-culture system and the differentiation of satellite cells. Such cells are ***not lipo-derived*** because they are derived from muscle tissue. Moreover, satellite cells are ***not stem cells*** because they are predetermined precursor cells (see, e.g., Young et al., page 202, column 2).
Hui-ling Su et al.	Discloses experiments conducted using embryonic mouse fibroblasts, ***not stem cells***
Marko et al.	A cell line isolated from bone marrow, ***not lipo-derived***
Sorisky et al.	Discloses the differentiation of preadipocytes. The document notes that preadipocytes are "committed to adipocyte lineage" (e.g., page 10, Fig 1); therefore such cells are ***not stem cells***.

Reference	Disclosure
U.S. Patent 5,486,359	Stem cells obtained from bone marrow, blood, dermis, periosteum, yolk sac, placenta, or umbilical cord (see column 1, lines 22-34, column 2, lines 18-21). As none of these tissues are adipose tissues, the stem cells described in this patent are ***not lipo derived***.
U.S. Patent 5,728,739 U.S. Patent 5,827,897	A method of differentiating preadipocytes, which are committed adipocyte precursor cells (see Sorisky et al., page 10) and therefore ***not stem cells***.
U.S. Patent 5,827,740 WO 98/04682	Discloses the differentiation of stem cells into adipose tissue. The stem cells, however, were derived from bone marrow and therefore ***not lipo-derived***.
Young et al.	Suggests the existence of stem cells in many embryonic tissues (see, e.g., table 1, page 139). The article does not identify adipose tissue as being one of those tissues, however. Thus, the article ***does not disclose lipo-derived stem cells***.

Thus, none of these references discloses the existence of stem cells within adipose tissue, nor do they disclose the isolation of stem cells from such tissue. As such, none of them discloses the subject matter of claim 1, nor any claims dependent thereon.

Discussion of Inventive Step

The Office Action alleges that claims 1-4, 15-16, and 77-79 lack an inventive step in light of the primary references discussed above in combination with either Considine et al., Hauner et al., Hausman et al., Shillabeer et al., or Vassaux et al. As is the case with respect to the primary references, none of these secondary references discloses a stem cell isolated or derived from adipose tissue. Thus, none of the cited references, alone or in combination, places the inventive lipo-derived stem cells within the state of the art. As such, the claims are inventive in light of all cited references.

Conclusion

The application is considered to be in good and proper form, and the Examiner is respectfully urged to indicate the acceptability of the present patent application in the International Preliminary Examination Report. If, in the opinion of the Examiner, a telephone conference would be of assistance in considering the subject application, the Examiner is invited to contact the undersigned by telephone.

Respectfully submitted,

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Date: 18 May, 2001

Substitute sheets follow

79. The kit of claim 78, wherein the medium is selected from the group of media consisting of adipogenic, chondrogenic, cardiogenic, dermatogenic, embryonic, fetal, hematopoetic, hemangiogenic, myogenic, nephrogenic, neurogenic, neuralgiagenic, urogenitogenic, osteogenic, pericardiogenic, peritoneogenic, pleurogenic, and splanchogenic, and stromogenic media.

80. A mammalian lipo-derived stem cell substantially free of mature adipocytes, which can be cultured in DMEM + about 10% fetal bovine serum without differentiating and which has two or more developmental phenotypes selected from the group of developmental phenotypes consisting of adipogenic, chondrogenic, cardiogenic, dermatogenic, hematopoetic, hemangiogenic, myogenic, nephrogenic, neurogenic, neuralgiagenic, urogenitogenic, osteogenic, pericardiogenic, peritoneogenic, pleurogenic, splanchogenic, and stromal developmental phenotypes.

81. The cell of claim 80, which can be cultured in DMEM + about 10% fetal bovine serum for at least 15 passages without differentiating

82. The cell of claim 80, which is human.

83. The cell of claim 80, which is genetically modified.

84. The cell of claim 80, which has a cell-surface bound intercellular signaling moiety.

85. The cell of claim 80, which secretes a hormone.

86. The cell of claim 85, wherein the hormone is selected from the group of hormones consisting of cytokines and growth factors.

87. A defined cell population comprising a cell of claim 80.

88. The defined cell population of claim 87, which is heterogeneous.

89. The defined cell population of claim 88, further compressing a stem cell selected from the group of cells consisting of neural stem cells (NSC), hematopoetic stem cells (HPC), embryonic stem cells (ESC) and mixtures thereof.

90. The defined cell population of claim 87, which consists essentially of cells according to claim 80.

91. The defined cell population of claim 87, which is substantially homogenous.

92. The defined cell population of claim 91, which is clonal.

93. A composition comprising the cell of claim 80 and a biologically compatible lattice.

94. A composition comprising the population of claim 87 and a biologically compatible lattice.

95. The composition of claim 94, wherein the lattice comprises polymeric material.

96. The composition of claim 95, wherein the polymeric material is formed of polymer fibers as a mesh or sponge.

97. The composition of claim 95, wherein the polymeric material comprises monomers selected from the group of monomers consisting of glycolic acid, lactic acid, propyl fumarate, caprolactone, hyaluronan, hyaluronic acid and combinations thereof.

98. The composition of claim 95, wherein the polymeric material comprises proteins, polysaccharides, polyhydroxy acids, polyorthoesters, polyanhydrides, polyphosphazenes, synthetic polymers or combinations thereof.

99. The composition of claim 95, wherein the polymeric material is a hydrogel formed by crosslinking of a polymer suspension having the cells dispersed therein.

100. The composition of claim 95, wherein the lattice further comprises a hormone selected from the group of hormones consisting of cytokines and growth factors.

101. A method of obtaining a genetically-modified cell comprising exposing the cell of claim 80 to a gene transfer vector comprising a nucleic acid including a transgene, whereby the nucleic acid is introduced into the cell under conditions whereby the transgene is expressed within the cell.

102. The method of claim 101, wherein the transgene encodes a protein conferring resistance to a toxin.

103. A method of delivering a transgene to an animal comprising (a) obtaining a genetically-modified cell in accordance with claim 102 and (b) introducing the cell into the animal, such that the transgene is expressed *in vivo*.

104. A method of differentiating the cell of claim 80, comprising culturing the cell in a morphogenic medium under conditions sufficient for the cell to differentiate.

105. The method of claim 104, wherein the medium is an adipogenic, chondrogenic, cardiogenic, dermatogenic, embryonic, fetal, hematopoetic, hemangiogenic, myogenic, nephrogenic, neurogenic, neuralgiagenic, urogenitogenic, osteogenic, pericardiogenic, peritoneogenic, pleurogenic, and splanchogenic, or stromogenic media.

106. The method of claim 104, wherein the morphogenic medium is an adipogenic medium and the cell is monitored to identify adipogenic differentiation.

107. The method of claim 104, wherein the morphogenic medium is a chondrogenic medium and the cell is monitored to identify chondrogenic differentiation.

108. The method of claim 104, wherein the morphogenic medium is an embryonic or fetal medium and the cell is monitored to identify embryonic or fetal phenotype.

109. The method of claim 104, wherein the morphogenic medium is a myogenic medium and the cell is monitored to identify myogenic differentiation.

110. The method of claim 104, wherein the morphogenic medium is an osteogenic medium and the cell is monitored to identify osteogenic differentiation.

111. The method of claim 104, wherein the morphogenic medium is a stromal medium and the cell is monitored to identify stromal or hematopoetic differentiation.

112. The method of claim 104, wherein the cell differentiates *in vitro*.

113. The method of claim 104, wherein the cell differentiates *in vivo*.

114. A method of producing hormones, comprising (a) culturing the cell of claim 85 within a medium under conditions sufficient for the cell to secrete the hormone into the medium and (b) isolating the hormone from the medium.

115. A method of promoting the closure of a wound within a patient comprising introducing the cell of claim 85 into the vicinity of a wound under conditions sufficient for the cell to produce the hormone, whereby the presence of the hormone promotes closure of the wound.

116. A method of promoting neovascularization within tissue, comprising introducing the cell of claim 85 into the tissue under conditions sufficient for the cell to produce the hormone, whereby the presence of the hormone promotes neovascularization within the tissue.

117. The method of claim 116, wherein the tissue is within an animal.

118. The method of claim 116, wherein the tissue is a graft.

119. The method of claim 116, wherein the hormone is a growth factor selected from the group of growth factor consisting of human growth factor, nerve growth factor, vascular and endothelial cell growth factor, and members of the TGFβ superfamily.

120. A method of conditioning culture medium comprising exposing a cell culture medium to the cell of claim 80 under conditions sufficient for the cell to condition the medium.

121. The method of claim 120, wherein the medium is separated from the cell after it has been conditioned.

122. A conditioned culture medium produced in accordance with the method of claim 120.

123. The conditioned culture medium of claim 122, which is substantially free of lipo-derived stem cells.

124. A method of culturing a stem cell comprising maintaining a stem cell in the conditioned medium of claim 122 under conditions for the stem cell to remain viable.

125. The method of claim 124, which further comprises permitting successive rounds of mitotic division of the stem cell to form an expanded population of stem cells.

126. The method of claim 124, wherein the medium is substantially free of lipo-derived stem cells.

127. The method of claim 124, wherein the medium contains lipo-derived cells.

128. The method of claim 127, wherein a stem cell and a lipo-derived cell are in contact.

129. The method of claim 124, wherein a stem cell is a hemopoetic stem cell.

130. An implant comprising the cell of claim 80.

131. An implant comprising the population of claim 87.

132. A method of isolating stem cells from adipose tissues comprising isolating adipose tissue from a patient and separating stem cells from the remainder of the adipose tissue.

133. The method of claim 132, further comprising differentiating the stem cells.

134. The method of claim 133, wherein the stem cells are differentiated into one or more precursor cell types.

135. The method of claim 134, wherein one or more precursor cell types is selected from the group of precursor cell types consisting of preadipocytes, premyocytes, and preosteocytes.

136. The method of claim 133, wherein the stem cells are differentiated into one or more mature cell types.

137. The method of claim 134, wherein one or more cell types is selected from the group of cell types selected from the group of cell types consisting of adipocytes, chondrocytes, dermal connective tissue cells, hemangial cells tissues, myocytes, osteocytes, neurons, neralglia, urogenital cells, pleural and peritoneal cells, visceral cells, mesodermal glandular cells, and stromal cells.

138. The method of claim 132, wherein the adipose tissue is liposuction effluent.

ABSTRACT

The present invention provides adipose-derived stem cells and lattices. In one aspect, the present invention provides a lipo-derived stem cell substantially free of adipocytes and red blood cells and clonal populations of connective tissue stem cells. The cells can be employed, alone or within biologically-compatible compositions, to generate differentiated tissues and structures, both *in vivo* and *in vitro*. Additionally, the cells can be expanded and cultured to produce hormones and to provide conditioned culture media for supporting the growth and expansion of other cell populations. In another aspect, the present invention provides a lipo-derived lattice substantially devoid of cells, which includes extracellular matrix material from adipose tissue. The lattice can be used as a substrate to facilitate the growth and differentiation of cells, whether *in vivo* or *in vitro*, into anlagen or even mature tissues or structures.

79. The kit of claim 78, wherein the medium is selected from the group of media consisting of adipogenic, chondrogenic, cardiogenic, dermatogenic, embryonic, fetal, hematopoetic, hemangiogenic, myogenic, nephrogenic, neurogenic, neuralgiagenic, urogenitogenic, osteogenic, pericardiogenic, peritoneogenic, pleurogenic, and splanchogenic, and stromogenic media.

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89. The defined cell population of claim 88, further compressing a stem cell selected from the group of cells consisting of neural stem cells (NSC), hematopoetic stem cells (HPC), embryonic stem cells (ESC) and mixtures thereof.

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93. A composition comprising the cell of claim 80 and a biologically compatible lattice.

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95. The composition of claim 94, wherein the lattice comprises polymeric material.

96. The composition of claim 95, wherein the polymeric material is formed of polymer fibers as a mesh or sponge.

97. The composition of claim 95, wherein the polymeric material comprises monomers selected from the group of monomers consisting of glycolic acid, lactic acid, propyl fumarate, caprolactone, hyaluronan, hyaluronic acid and combinations thereof.

98. The composition of claim 95, wherein the polymeric material comprises proteins, polysaccharides, polyhydroxy acids, polyorthoesters, polyanhydrides, polyphosphazenes, synthetic polymers or combinations thereof.

99. The composition of claim 95, wherein the polymeric material is a hydrogel formed by crosslinking of a polymer suspension having the cells dispersed therein.

100. The composition of claim 95, wherein the lattice further comprises a hormone selected from the group of hormones consisting of cytokines and growth factors.

101. A method of obtaining a genetically-modified cell comprising exposing the cell of claim 80 to a gene transfer vector comprising a nucleic acid including a transgene, whereby the nucleic acid is introduced into the cell under conditions whereby the transgene is expressed within the cell.

102. The method of claim 101, wherein the transgene encodes a protein conferring resistance to a toxin.

103. A method of delivering a transgene to an animal comprising (a) obtaining a genetically-modified cell in accordance with claim 102 and (b) introducing the cell into the animal, such that the transgene is expressed *in vivo*.

104. A method of differentiating the cell of claim 80, comprising culturing the cell in a morphogenic medium under conditions sufficient for the cell to differentiate.

105. The method of claim 104, wherein the medium is an adipogenic, chondrogenic, cardiogenic, dermatogenic, embryonic, fetal, hematopoetic, hemangiogenic, myogenic, nephrogenic, neurogenic, neuralgiagenic, urogenitogenic, osteogenic, pericardiogenic, peritoneogenic, pleurogenic, and splanchogenic, or stromogenic media.

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108. The method of claim 104, wherein the morphogenic medium is an embryonic or fetal medium and the cell is monitored to identify embryonic or fetal phenotype.

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110. The method of claim 104, wherein the morphogenic medium is an osteogenic medium and the cell is monitored to identify osteogenic differentiation.

111. The method of claim 104, wherein the morphogenic medium is a stromal medium and the cell is monitored to identify stromal or hematopoetic differentiation.

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117. The method of claim 116, wherein the tissue is within an animal.

118. The method of claim 116, wherein the tissue is a graft.

119. The method of claim 116, wherein the hormone is a growth factor selected from the group of growth factor consisting of human growth factor, nerve growth factor, vascular and endothelial cell growth factor, and members of the TGFβ superfamily.

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121. The method of claim 120, wherein the medium is separated from the cell after it has been conditioned.

122. A conditioned culture medium produced in accordance with the method of claim 120.

123. The conditioned culture medium of claim 122, which is substantially free of lipo-derived stem cells.

124. A method of culturing a stem cell comprising maintaining a stem cell in the conditioned medium of claim 122 under conditions for the stem cell to remain viable.

125. The method of claim 124, which further comprises permitting successive rounds of mitotic division of the stem cell to form an expanded population of stem cells.

126. The method of claim 124, wherein the medium is substantially free of lipo-derived stem cells.

127. The method of claim 124, wherein the medium contains lipo-derived cells.

128. The method of claim 127, wherein a stem cell and a lipo-derived cell are in contact.

129. The method of claim 124, wherein a stem cell is a hemopoetic stem cell.

130. An implant comprising the cell of claim 80.

131. An implant comprising the population of claim 87.

132. A method of isolating stem cells from adipose tissues comprising isolating adipose tissue from a patient and separating stem cells from the remainder of the adipose tissue.

133. The method of claim 132, further comprising differentiating the stem cells.

134. The method of claim 133, wherein the stem cells are differentiated into one or more precursor cell types.

135. The method of claim 134, wherein one or more precursor cell types is selected from the group of precursor cell types consisting of preadipocytes, premyocytes, and preosteocytes.

136. The method of claim 133, wherein the stem cells are differentiated into one or more mature cell types.

137. The method of claim 134, wherein one or more cell types is selected from the group of cell types selected from the group of cell types consisting of adipocytes, chondrocytes, dermal connective tissue cells, hemangial cells tissues, myocytes, osteocytes, neurons, neralglia, urogenital cells, pleural and peritoneal cells, visceral cells, mesodermal glandular cells, and stromal cells.

138. The method of claim 132, wherein the adipose tissue is liposuction effluent.